

A human being is a whole world to a mitochondrion, just the way our planet is to us. But we're much more dependent on our mitochondria than the earth is on us.

Madeleine L'Engle, *A Wind in the Door* (1973)

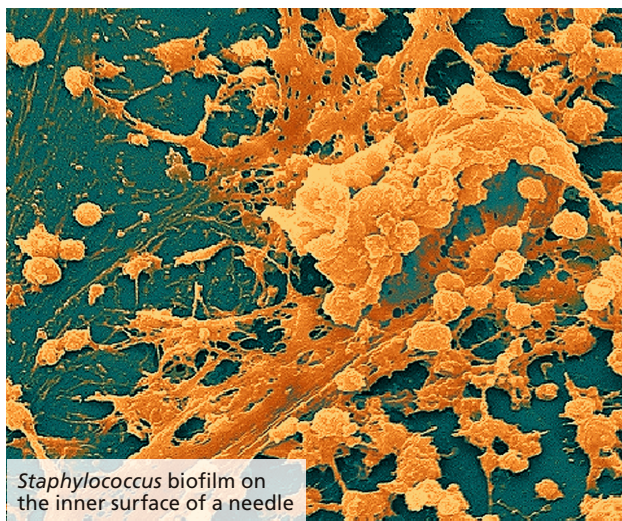
## ANTIBIOTIC RESISTANCE

# Biofilm Dispersing Agent Rejuvenates Older Antibiotics

An estimated 75% of bacterial infections involve biofilms, surface-attached colonies of bacteria that are protected by an extracellular matrix.<sup>1</sup> Bacteria protected within biofilms are up to 1,000 times more resistant to antibiotics than if they were free-floating (planktonic),<sup>2</sup> which severely complicates treatment options. Rather than searching for better antibiotics, researchers have discovered that small molecules<sup>3</sup> known as 2-amino-imidazoles disrupt biofilms, making antibiotic-resistant strains of bacteria more vulnerable to conventional drugs.<sup>4</sup> Moreover, antibiotics enhance the ability of 2-amino-imidazoles to disrupt biofilms. "Perhaps new antibiotics are not the only way to combat biofilm infections if we could make ineffective older antibiotics active again," says principal investigator Christian Melander, an associate professor of bio-organic chemistry at North Carolina State University.

Melander and his colleagues started with natural 2-amino-imidazoles (isolated from sponges) including oroidin and age-liferin, which are known to block biofilm formation. They synthesized an improved version of oroidin, 2-amino-imidazole/triazole (2-AIT), which featured more powerful antibiofilm activity and less toxicity to surrounding human cells and organs.<sup>5</sup> Although 2-AIT alone does not kill bacteria, it disperses biofilms, releasing planktonic cells that are more susceptible to antibiotics than bacterial cells shielded by sticky biofilms.

The researchers tested 2-AIT against a variety of clinically relevant biofilm infections along with antibiotics that currently are used or have been used in the past to treat them. Multidrug-resistant strains of *Acinetobacter baumannii* plague soldiers wounded in the Middle East, and colistin, an older antibiotic with toxic side effects, remains a treatment of last resort due to extensive side effects.<sup>6</sup> *Staphylococcus aureus* infections that colonize catheters and other indwelling medical devices were treated with the antibiotic novobiocin until drug-resistant *S. aureus* strains arose.<sup>7</sup> Tobramycin is an inhaled antibiotic currently used to treat



*Staphylococcus* biofilm on the inner surface of a needle

*Pseudomonas aeruginosa* infections that clog the lungs of cystic fibrosis patients.<sup>8</sup> "We chose three antibiotics known to act against certain bacteria circulating in hospital settings that have become drug resistant," says Melander.

The biofilms were grown in culture, then treated with their corresponding antibiotic, all of which produced little dispersion. However, the addition of 2-AIT to the antibiotics produced dramatic dispersion of up to 1,000-fold.<sup>4</sup> Like adjuvants that boost the power of vaccines, 2-AIT is "our version of a small molecule adjuvant that allows several classes of older antibiotics to work again," says Melander. He suspects 2-AIT somehow short-circuits bacterial signaling pathways that regulate biofilm formation; experiments are under way to unravel the details.

2-AIT also was shown to resensitize drug-resistant bacteria to death by antibiotic. When a clinical strain of methicillin-resistant *S. aureus* (MRSA) was treated with 2-AIT alone, it grew normally. The addition of methicillin, however, reduced its growth by 99%. Additionally, 2-AIT lowered the amount of antibiotics needed to inhibit bacterial growth.<sup>4</sup>

The combination of 2-AIT with antibiotics could serve as a parallel treatment for antibiotic-resistant infections. The results suggest this cooperative approach may enable "obsolete antibiotics to overcome infections that otherwise would persist if treated with either agent individually," says Melander. A small molecule adjuvant like 2-AIT potentially

could be given orally in pill form, he says.

2-AIT "most remarkably can actually disperse preformed biofilms, something that is much more difficult to do than simply inhibiting their formation," says Neville Kallenbach, a professor of chemistry at New York University in New York City. Because biofilms are much harder to kill than planktonic bacteria, the combination therapy opens a new avenue for remediating persistent biofilm infections. "The ability to disperse bio-

films formed by multidrug-resistant bacteria adds a major new weapon to the limited arsenal of therapies available today," says Kallenbach—and the impact on human health could be enormous.

Agents such as 2-AIT also lend themselves to solving environmental biofilm problems including the biofouling of ship hulls and plugging of waterlines by microbes such as *Escherichia coli* and *Bacillus*, *Pseudomonas*, *Proteobacter*, and *Actinobacteria* species. Today's antifouling paints contain copper, which leaches into seawater, where it inhibits enzymatic activity in brine shrimp larvae<sup>9</sup> and impairs sperm quality and larvae development in sea urchins,<sup>10</sup> among other effects. Melander is working on a copper-free 2-AIT-based polymer spray to prevent biofouling.

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## CHEMICAL EXPOSURES

## More Iodine or Less Perchlorate?

Perchlorate is believed to block uptake of iodine into the thyroid, eventually resulting in the decreased production of the thyroid hormones thyroxine and triiodothyronine. But a science review of perchlorate concludes that reducing the risk of mental deficits in children whose mothers are exposed to the chemical may be achieved most efficiently by correcting the iodine deficiency that occurs in roughly a third of U.S. women of child-bearing age—not by reducing perchlorate intake.<sup>1</sup>

The review is a first for the U.S. Environmental Protection Agency (EPA) Office of Inspector General (OIG), which primarily conducts audits, evaluations, and investigations of the EPA and its contractors to promote economy and efficiency, and to prevent and detect fraud, waste, and abuse. But rather than resolving controversy over the risk characterization of perchlorate, the review appears instead to be further fueling it.<sup>2</sup>

In comments offered in response to the review, the Environmental Working Group wrote that the OIG had used the review to justify their endorsement of the Bush Administration's failure to set a drinking water standard for perchlorate, which pollutes the drinking water of an estimated 20–40 million people nationwide.<sup>3</sup> But Purnendu Dasgupta, an analytical chemist at the University of Texas, Arlington, applauds the OIG for stepping in to address a major public health gap. “The continued brouhaha about perchlorate alone, whether by activists or protectionists, merely acts as a smokescreen,” he says. “We have urgent problems about iodine nutrition; the preoccupation with perchlorate alone is obscuring the fact that we are gambling with the intellectual future of the next generation at our peril.”

Perchlorate is thought to affect thyroid function by blocking uptake of iodine, an essential component of thyroid hormones, which orchestrate brain development. Other chemicals—in particular, thiocyanate (found in tobacco smoke and cruciferous vegetables) and nitrate (found in leafy vegetables, processed meats, and some contaminated water supplies)—act in a similar way. Too little iodide also has the same effect. The OIG considered all four of these factors in its cumulative risk assessment, a type of assessment that looks at the public health risk arising from multiple, combined stressors.

By attempting a more holistic cumulative assessment, OIG says it is at the vanguard of governmental agencies in following recommendations from several recent

governmental advisory committees.<sup>2</sup> House and Senate draft versions of chemical regulation reform bills also call for cumulative risk assessments. Risk assessment specialists generally applaud this innovative aspect of the OIG effort. But many comments on the report referred to the lack of peer review, failure to consider major studies, failure to specifically consider the risk of perchlorate exposure to infants, and an excessive reliance on one *in vitro* study<sup>4</sup> that estimated the relative potencies of the different thyroid stressors in terms of their ability to block iodine uptake.

The OIG hired consultancy ICF International to conduct a technical review of the assessment. ICF International broadly endorsed the OIG's cumulative risk assessment approach, but recommended the use of more recent peer-reviewed human studies, in particular a 2006 study<sup>5</sup> that found a statistically significant association between changes in thyroid function to levels of perchlorate exposure roughly an order of magnitude lower than those in previous studies of people exposed to perchlorate.

The Environmental Working Group contends ICF International had a potential conflict of interest because the firm has consulted for federal agencies, military contractors, and other entities responsible for perchlorate pollution in drinking water supplies, all of whom “have vigorously opposed strong public health standards for perchlorate.”<sup>3</sup> The Massachusetts Department of Environmental Protection raised similar concerns. But the OIG contends ICF International was selected as the best qualified bidder under federal guidelines.<sup>2</sup>

Other questions revolve around data suggesting perchlorate may have additional mechanisms of action beyond its ability to inhibit iodine uptake.<sup>6</sup> “Although the OIG study is informative with respect to cumulative impacts at the level of thyroidal iodine uptake, the potential existence of additional mechanisms of action should temper conclusions regarding appropriate perchlorate exposure limits, especially where the iodine uptake inhibition estimates are derived from an *in vitro* model that does not reflect the complexity of *in vivo* thyroid function, effects, and responses,” says toxicologist C. Mark Smith of the Massachusetts Department of Environmental Protection.

Adam Finkel, a member of the National Research Council committee that evaluated EPA risk assessment protocols,<sup>7</sup> notes moreover that cumulative risk assessments such as this could end up yielding questionable policy. “Advocates for holistic risk assessments assumed the point to be that you can make a stronger case for reducing pollutant

X if you see it in context of all the other things also adding to the burden of disease Y—but this report turns that logic on its head and says essentially that when you see the whole picture, you see a reason to ignore the pollutant and work on the other things,” he explains.

The conclusions of the review conflict with risk assessments conducted by states such as California and Massachusetts, which have adopted health recommendations more stringent than the current EPA reference dose for perchlorate of 0.0007 mg/kg/day (total intake from both water and food). “Although improving iodine nutrition is an important public health issue itself, it is an incomplete response to perchlorate drinking water contamination,” Smith says. “Infants are the population of greatest concern identified in the Massachusetts risk assessment, but the OIG assessment doesn't adequately address their demonstrated potential for significant perchlorate exposure and risk.”

“It's great that this cumulative assessment looks more broadly and seeks to consider possible risk management solutions early in the assessment process,” says Finkel. “But while adding iodide may be the most efficient solution, that is not for the risk assessor to prejudge—we need a document that lays out the costs and benefits of alternative approaches, not one that trivializes the environmental risk because there may be a ‘supply side’ way of sidestepping it.”

Jonathan Levy, who also was a member of the panel that evaluated EPA risk assessment protocols,<sup>7</sup> agrees. “Our NAS committee recommendations would argue that the presence of multiple stressors would imply that health effects would be anticipated at low dose of perchlorate,” he says. “The fact that other stressors have greater effects is an interesting observation, but we explicitly stated that this should not be the primary output of cumulative risk assessment.”

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## CANCER

# First Combined Analysis from INTERPHONE Inconclusive

Long-awaited results of the largest effort yet to investigate whether cell phone use contributes to brain cancers are finally available.<sup>1</sup> But the May report, the first combined analysis of results from the International Agency for Research on Cancer's (IARC) \$24-million INTERPHONE study, is inconclusive, stating that "suggestions of an increased risk of glioma at the highest exposure levels, but biases and errors prevent a causal interpretation."

The interview-based case-control INTERPHONE study was the collaborative effort of 48 researchers from 13 nations. It began in 2000 and included more than 14,000 participants, among them 2,765 glioma and 2,425 meningioma cases and matched controls<sup>2</sup> (the current analysis included 2,708 glioma and 2,409 meningioma cases). No other studies have included as many exposed cases, particularly of long-term and heavy users of cell phones.

A major challenge the researchers faced in interpreting the data was the high refusal rates among controls—that is, controls were successfully contacted but declined to give the information sought—says the study's principal investigator, Elisabeth Cardis, now of the Centre for Research in Environmental Epidemiology in Barcelona, Spain. "This resulted in mobile phone users being overrepresented among controls," Cardis explains. The vast majority of the study's risk estimates are below 1, which suggests there might have been a selection bias in amassing the study population, she says.

Additionally, cell phone usage patterns have changed significantly in the decade since INTERPHONE began. "Most of the users in the study had relatively low use compared to today's use," Cardis points out. The usage by people in the study's highest cumulative call time group corresponds to about half an hour a day for a period of 10 years



In the most recent industry figures from CTIA,<sup>7</sup> U.S. cell phone users logged about 2.3 trillion minutes of use per year, but many users are reporting an increase in text messages over calls.

or more, which is "pretty normal or even light use today," she says. At the same time, concerns over recall bias also made the data hard to interpret. For example, some cases—but no controls—claimed to spend 12 or more hours a day on their cell phone.

Besides the brain tumors assessed in the current study, INTERPHONE also evaluated correlations between cell phone use and tumors of the acoustic nerve and the parotid salivary gland. These two tumor types will be the focus of future reports, Cardis says.

The period of exposure for all of the subjects included in INTERPHONE is relatively short for assessing a causative link to a cancer, according to a commentary published alongside the study.<sup>3</sup> Cell phone use began in the 1980s but was not widespread until the mid-1990s, wrote authors Rodolfo Saracci of Italy's National Research Council in Pisa and Jonathan Samet of the University of Southern California's Department of Preventive Medicine. "None of . . . today's established carcinogens, including tobacco, could have been firmly identified as increasing risk in the first 10 years or so since

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## The Beat by Erin E. Dooley

### EWG Issues 2010 Sunscreen Guide

In its fourth report on sunscreen products, the Environmental Working Group recommends only 8% of 500 products tested.<sup>1</sup> The group reports a surge in products boasting an SPF higher



Contrary to public perception, there is no evidence sunscreen prevents skin cancer.

than 50, "which sell a false sense of security" since higher SPF does not necessarily equate to more protection. Several products contained ingredients of potential health concern: retinyl palmitate, which has been linked to accelerated development of skin tumors and lesions, was found in 41% of sunscreens assessed, and oxybenzone, an endocrine-disrupting compound, was found in 60%.

### EPA Exposure Assessment: PBDEs

A new EPA exposure assessment<sup>2</sup> shows that U.S. exposure to polybrominated diphenyl ethers (PBDEs) occurs primarily through house dust, unlike other persistent organic pollutants, which typically are encountered in food. Additionally, weight-specific intake rates are higher for U.S. children, especially infants, than for adults. The EPA is planning to issue new rules later this year for the manufacture and import of products containing two specific PBDEs. PBDE flame retardants, some of which have already been phased out of commerce, are used in applications including furniture and electronics.

"Safer" cigarettes?  
Nice try, but no cigar.



### "Safer" Cigarettes Still Hazardous

Smoking tobacco- and nicotine-free cigarettes made of lettuce may be at least as hazardous as smoking conventional tobacco cigarettes, if not more so. In a study of the supposedly safer cigarettes, which were introduced in 1997, dose-dependent double-strand DNA breaks were seen after shorter durations of exposure to smoke compared with conventional cigarettes.<sup>3</sup>

Left to right: iStockphoto.com; Shutterstock

first exposure,” explained Saracci and Samet. “Ionizing radiation is a recognized cause of brain tumors, but except for rare instances the radiation-induced cases occur on average after 10–20 years since the time of first exposure.” The authors conclude, therefore, that “observing no increase in risk would be reassuring but only to a limited extent.”

Publication of the first results from INTERPHONE was originally expected in 2006. Cardis says the report was delayed because of the large research team’s difficulties in interpreting the results. “The entire study group and all of the coauthors . . . spent a lot of time conducting hundreds of additional analyses, reviewing the analyses, and trying to understand the potential biases of the study,” she says. “We’ve conducted about every analysis that we could think to do.”

One of the analyses that did not make it into the main text of the report is Appendix 2, which is mentioned in Saracci and Samet’s commentary. Published only online as supplementary material, it presents an alternative analysis that suggests an increase in glioma among subjects in the top 10% of cumulative call time. The alternative analysis compared the incidence of glioma in the most highly exposed subjects to that in study subjects who had the lowest amount of exposure among regular cell phone users. In contrast, the primary analysis compared the incidence of glioma in the highly exposed group to the incidence among subjects who reported that they rarely or never used cell phones at all.

This approach—which accounts for the possibility that cell phone radiation exposure is not the only potential risk factor that differs between people who regularly use cell phones and people who don’t—is common in occupational epidemiology. However, some INTERPHONE investigators believed the analysis would be inappropriate if the main reason for the decreased odds ratios observed in the study was not selection bias. “We have legitimate differences in the interpretation of these results and the value of this analysis,” Cardis says.

IARC director Christopher Wild says, “Observations at the highest level of cumulative call time and the changing patterns of mobile phone use since the period studied by INTERPHONE, particularly in young people, mean that further investigation of mobile phone use

and brain cancer risk is merited.” John Walls, vice president of public affairs for CTIA-The Wireless Association®, which represents the cell phone industry, says, “The possible effects of long-term heavy use of mobile phones require further investigation.”

Three important new studies are already under way to collect more data. The first is an animal study being conducted by the National Toxicology Program to assess the effects of long-term exposure to radiofrequency energy in rats and mice.<sup>4</sup> The study allows for precise control over the exposure, as well as a “thorough evaluation for the presence of tumors, not just of the brain, but throughout the entire body,” says program associate director John Bucher.

The other two studies are epidemiologic. The case–control MOBI-KIDS study was launched last year in 13 countries to investigate potential risk factors for brain tumors in children, including cell phone use.<sup>5</sup> Children’s rates of brain cancers have been rising in recent years, according to the study’s organizers, who hope to recruit approximately 2,000 brain cancer patients and matched controls. The COSMOS cohort study, launched in April with the specific goal of studying health effects of cell phone use, aims to recruit more than 250,000 people in five European countries and follow them for up to 30 years.<sup>6</sup>

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The tobacco- and nicotine-free cigarettes also delivered far higher doses of total particulate matter (“tar”). The researchers used phospho-specific antibodies to measure DNA damage response and their own laser scanning cytometry instrumentation, which they say should be a useful complement to other methods for assessing genotoxicity of cigarette smoke.

## BPA and Male Sexual Dysfunction

Bisphenol A (BPA) is used in a large number of consumer products, including plastic containers and food and beverage can linings. Following up on an earlier study<sup>4</sup> comparing workers with and without occupational BPA exposure, researchers assessed urine BPA levels and sexual function in a subset of workers and found that increasing urine BPA level was associated with decreasing values for seven measures of sexual function.<sup>5</sup> An additional analysis restricted to workers exposed to BPA only nonoccupationally revealed a similar trend, but the authors wrote that “many of the estimates were no longer statistically significant due to the markedly reduced sample size.”

## Indoor Tanning and Melanoma: Evidence Strengthens

A new study presents strong evidence that use of tanning beds may lead to higher odds of melanoma.<sup>6</sup> Compared with people who never tanned indoors, people using any tanning bed were almost 75% more likely to develop melanoma, and frequent users

of indoor tanning beds had the highest risk. The study also showed for the first time that melanoma was more strongly associated with frequency of tanning than with age at which indoor tanning began. Earlier studies showed only weak associations with melanoma risk; most were unable to adjust for sun exposure or did not confirm dose response or compare specific tanning devices—gaps bridged in the current population-based case–control study. Melanoma, the most dangerous form of skin cancer, is also one of the fastest increasing cancers in the United States.<sup>7</sup>



An advisory panel to the FDA has recommended restrictions on the use of tanning beds by teenagers.

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